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ABSTRACT

Background: The cardiopulmonary effects of the individual criteria air pollutants have been well investigated, but little is known about the cardiopulmonary effects of inhaled multi-pollutant-mixtures that more realistically represent environmental exposures.

Objectives: This study was designed to assess the cardiopulmonary effects of exposure to photochemically altered particle-free multi-pollutant-mixtures.

Methods: Mice were exposed to: filtered air (FA), multi-pollutant-mixtures or O_3 for 4 hr in a photochemical reaction chamber. Eight hr after exposure, cardiac responses were assessed using a Langendorff preparation using a protocol consisting of 20 min of global ischemia followed by 2 hr of reperfusion. Cardiac function was measured by index of left-ventricular developed pressure (LVDP) and contractility (dP/dt) before ischemia. On reperfusion after ischemia, recovery of post-ischemic LVDP and infarct size were examined. Bronchoalveolar lavage (BAL) cell counts were used to assess lung inflammation.

Results: Exposure to the multi-pollutant-mixtures decreased LVDP, dP/dt_{max} and dP/dt_{min} compared to FA. Exposure to O_3 also decreased heart rate and dP/dt_{min}. Time to ischemic contracture was prolonged in the multi-pollutant-mixtures group relative to that in the FA. Mice in the multi-pollutant-mixtures group had better recovery of post-ischemic LVDP and smaller infarct size. The multi-pollutant-mixtures and O_3 exposure increased numbers of macrophages in the BAL fluid.

Conclusions: This study demonstrates that acute multi-pollutant-mixtures inhalation decreases LVDP and cardiac contractility in isolated non-ischemic murine hearts, prolongs ischemic contracture, increases post-ischemic recovery of LVDP, and reduces infarct size, suggesting that

exposure to photochemically altered urban air pollution appears to affect cardiac mechanics in isolated perfused hearts.

INTRODUCTION

Epidemiological studies have linked acute and chronic ambient air pollution exposure with cardiovascular diseases and shown that air pollution exposure increases the risk of mortality, ischemic heart disease, heart failure, and arrhythmias (Brook et al. 2010). The Clean Air Act (www.epw.senate.gov/envlaws/cleanair.pdf) established National Air Quality Standards for individual "criteria pollutants", and consequently air pollution health effects research studies have largely focused on characterizing the effects of exposure to these pollutants on an individual basis. However, "real" world air pollution is far more complex than exposure to an individual agent, containing freshly emitted primary aerosol as well as photochemically aged secondary aerosols formed in the atmosphere during the oxidation of gas-phase precursors (Kanakidou et al. 2005). There is a growing realization that a multi-pollutant experimental approach is needed to understand the relevant modes of action of ambient air pollutant mixtures on human health (Dominici et al. 2010). However, there is currently a paucity of data on how complex mixtures impact key target organ systems associated with morbidity and mortality. In order to fill this critical knowledge gap, in this study we tested whether inhaled exposure to particle-free complex mixtures representative of gaseous mixtures found in urban environments can modulate pulmonary inflammation and cardiac mechanics.

We used an environmental photochemical reaction chamber located at the University of North Carolina at Chapel Hill to generate model multi-pollutant-mixtures atmospheres. The chamber uses sunlight to imitate the natural photochemistry of urban mixtures and produce a combination of compounds that is similar in composition to that found in urban multi-pollutant-mixtures (Jeffries 1995; Sexton et al. 2004). Previous *in vitro* studies have utilized these chambers to expose individual cell cultures to examine the toxicity of air pollution mixtures. Using this

approach, it has been shown previously that photochemically altered particle-free urban mixtures exposure causes significant inflammatory responses (Sexton et al. 2004) and greater genetic alterations (Rager et al. 2011) compared to primary urban mixtures.

Experimental studies have demonstrated the ability of individual air pollutants to cause cardiopulmonary toxicity in animals. For example, acute exposure to PM can increase pulmonary and systemic inflammation and lung injury, cause vascular dysfunction, alter heart rate variability, induce arrhythmia, and enhance cardiac ischemic injury (Brook et al. 2010). We (Cho et al. 2009; Tong et al. 2010) and others (Cozzi et al. 2006) have previously shown that PM exposure enhanced cardiac ischemia/reperfusion injury in animals. However, extrapolation to multi-pollutant-mixtures is far from simple. For instance, the redox cycling potential of a mixture, which is thought to be an important predictor of generation of inflammation, may be very different to that of its constituent parts. In order to address this issue, we tested the hypothesis that photochemically aged particle-free multi-pollutant-mixtures can cause inflammation and impair cardiac function. Using a murine model we evaluated the effects of multi-pollutant-mixtures exposure on cardiovascular and pulmonary endpoints. We report here that exposure to multi-pollutant-mixtures at concentrations that produce only minimal pulmonary effects significantly affect cardiac function including decreased left ventricular developed pressure and contractility, but unexpectedly reduced cardiac ischemia/reperfusion injury.

METHODS AND MATERIALS

Experimental Animals

All experimental procedures were performed in compliance with protocols approved by the University of North Carolina at Chapel Hill IACUC according to NIH guidelines. The animals

were treated humanely and with regard for alleviation of suffering. Mice were maintained at 22°C with a 12-hr light/dark cycle and free access to food (ProLab RMH 3000) and water. Forty-five female C57Bl/6 mice (5 month old, average weight 23.9 ± 0.39 gram) were purchased from Jackson Laboratory (Bar Harbor, ME) and acclimated for 2 months before they were used.

Generation of Photochemical Urban Mixtures

The University of North Carolina at Chapel Hill's outdoor environmental photochemical reaction chamber was used to generate exposure atmospheres. Synthetic urban mixture (Scott Specialty Gases, Plumsteadville, PA), a VOC mixture (Jeffries 1995), and NO_x (nitric oxide and nitrogen dioxide) were used as the starting materials for the test atmosphere. The synthetic particle-free urban mixture contains 55 different hydrocarbons at specific ratios that represent chemicals present in urban atmospheres (Sexton et al. 2004). On the morning of the exposure, the chamber was humidified naturally by pre-flushing with HEPA-filtered ambient air. At 0700 hr, the volatile organics of synthetic urban mixture were drawn from a gas cylinder into the photochemical reaction chamber while a liquid mixture containing less-volatile organics was injected into the chamber. NO_x was drawn from a gas cylinder (AirGas, National Welders, Morrisville, NC) into the chamber to establish a test atmosphere containing 2 ppm NO_x.

Chemical constituents inside the chamber during the experiment were assessed by gas measurement methods as described previously (Rager et al. 2011). NO and NO_2 levels were measured every min using a Teledyne model 9841 No_x analyzer (Teledyne Monitor Labs, Englewood, CO). O_3 was measured every min with a Teledyne model 9811 monitor. Concentrations of these compounds were averaged during the exposure. Other secondary products such as the carbonyl containing aldehydes and ketones were measured every 15 min by

gas chromatography and mass spectrometry. The O_3 level was elevated to 0.243 ppm in the chamber; therefore the same concentration of O_3 was used in the single pollutant O_3 exposure. O_3 was generated from oxidized air using an O_3 generator (Model OL80A, Ozone Services, Yanco Industries, Burton, BC).

Animal Exposure

As the photochemical reaction depends on the weather condition and the multi-pollutant-mixtures generated is not reproducible, mice were exposed in groups to photochemically aged particle-free multi-pollutant-mixtures (n = 15), 0.245 ppm of O_3 (*n* = 14), or filtered air (FA) (*n* = 16) for 4 hr (2000-2400 hr) during their dark cycle on three separate days in an outdoor photochemical reaction chamber. Under each exposure, one group of mice (*n* = 7 in the multi-pollutant-mixtures group; *n* = 6 in the O_3 group; *n* = 8 in the FA group) was used for the isolated heart perfusion and the other group of mice (*n* = 8 per group) was used for assessment of lung inflammation.

Cardiac Function

As described previously (Tong et al. 2009), eight to eleven hr after exposure, mice were anesthetized with an intraperitoneal injection of sodium pentobarbital (80 mg/kg body weight). After intravenous heparin (100 units) injection the hearts were rapidly excised and placed in ice-cold Krebs-Henseleit buffer. The aortas were cannulated and perfused retrograde at constant pressure of 100 cmH_2O. The non-recirculating perfusate was a Krebs-Henseleit buffer containing (in mmol/L) 120 NaCl, 5.9 KCl, 1.2 $MgSO_4$, 1.75 $CaCl_2$, 25 $NaHCO_3$, and 11 glucose. The buffer was aerated with 95% O_2 and 5% CO_2, and maintained at pH 7.4 and 37°C.

For assessment of contractile function, a latex balloon on the tip of a polyethylene catheter was inserted through the left atrium into the left ventricle. The catheter was connected to a pressure transducer (Argon Medical Devices, Athens, TX) at the same height as the heart. The pressure of the left ventricular balloon was inflated to 0-5 cmH_2O. A PowerLab system was used to collect and process the heart rate, left ventricular developed pressure [LVDP = LV peak minus end-diastolic pressure (LVEDP)], and contractility (dP/dt) data (AD Instruments, Milford, MA). All hearts were perfused for 25 min when the baseline measurements were taken prior to initiating 20 min of global no-flow ischemia followed by 2 hr of reperfusion. Onset of ischemic contracture was detected when the left ventricular pressure began to increase during ischemia. Recovery of LVDP, expressed as a percentage of the initial pre-ischemic LVDP was measured at 40 min of reperfusion after 20 min of ischemia.

Cardiac Necrosis Evaluation

As described previously (Tong, 2009; Tong, 2010), at the end of 2 hr of reperfusion, hearts were perfused with 15 ml of 1% solution of 2,3,5-triphenyltetrazolium chloride (TTC) dissolved in Krebs-Henseleit buffer, then incubated in 1% TTC at 37°C for 10 min, and then fixed in formalin. The area of necrosis was measured by taking cross-sectional slices through the ventricles, which were then photographed using a digital camera mounted on a stereo-microscope. The resulting images were quantified by measuring the areas of stained (viable tissue) versus unstained tissue (infarct) with the use of Adobe Photoshop. Infarct size was expressed as a percentage of the total ventricular section and averaged from four images.

Bronchoalveolar Lavage

Lung inflammation has been shown to be one of the pathways mediate the cardiac effects (Brook et al. 2010), therefore we examined whether multi-pollutant-mixtures or O_3 exposure resulted in lung inflammation in this study. Twelve hr after exposure, mice were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg). The lungs were lavaged five times with 1 mL of 1x Hanks' Balanced Salt Soultion (HBSS, Gibco) and cellular components of the bronchoalveolar lavage (BAL) fluid were separated by centrifugation at 1500 rpm for 10 min at 4^{o}C. The cell pellet was resuspended in 500 μL HBSS and total cells were counted using a hemocytometer. Cytospin preparations were made and stained with Hema 3 (Fisher Scientific) to evaluate the BAL cellular composition.

Statistical Analysis

Data are expressed as means ± SEM. Non-parametric analyses were performed as the data was not normally distributed. Comparisons among the multi-pollutant-mixtures, O_3, and FA control group were performed by Kruskal-Wallis U-test followed by Dunn's multiple comparison test. Mann-Whitney U-test was used to compare the multi-pollutant-mixtures or O_3 group with FA control group. The statistical significance levels was set at $p < 0.05$.

RESULTS

Composition of Particle-Free Photochemically Generated Multi-Pollutant-Mixtures

Photochemical reactions of the original hydrocarbons and NO_x mixtures generated more than 300 carbonyl secondary products. Among these products, the levels of detected VOCs compounds estimated in the chamber are listed in Table 1. As seen in a previous study (Rager et

al. 2011), average chamber NO_2 and NO levels decreased throughout the day, and levels of secondary chemical products, such as O_3, formaldehyde, and acetaldehyde increased. The photochemical chamber contained 0.243 ppm of O_3 and secondary carbonyls. The formaldehyde level in the photochemical chamber was five times higher than that in the O_3 and FA control chambers, and the acetaldehyde level was elevated in the photochemical chamber but not detectable in the O_3 and FA control chambers. No particulate matter or secondary organic aerosol was formed within the chamber.

Cardiac Effects

When compared to FA control, the baseline heart rate before ischemia decreased with O_3 exposure, whereas they were unchanged in the hearts of mice exposed to the particle-free multi-pollutant-mixtures (Figure 1A). No differences in baseline coronary artery flow rate at constant pressure were observed between the multi-pollutant-mixtures or O_3 groups compared to the FA control group (Table 2). However, hearts from the multi-pollutant-mixtures group had lower baseline LVDP (69.2 ± 16.0 cm H_2O) compared to FA group (146.4 ± 14.8 cm H_2O; $p < 0.05$), though there was no significant difference between the FA- and O_3-group (134.2 ± 5.4 cm H_2O) (Figure 1B). Exposure to multi-pollutant-mixtures or O_3 decreased baseline left ventricular contractility. The baseline rate of contraction (dP/dt_{max}) was lower in the multi-pollutant-mixtures group (2764 ± 558 cm H_2O/s) compared to the FA group (5405 ± 400 cm H_2O/s; $p < 0.05$) (Figure 2A). Yet, the change in dP/dt_{max} did not differ between the O_3 (4070 ± 704 cm H_2O/s) and FA group. The baseline rate of relaxation (dP/dt_{min}) was decreased by exposure to the multi-pollutant-mixtures (–1822 ± 335 cm H_2O/s; $p < 0.01$) and O_3 exposure (–2477 ± 407 cm H_2O/s; $p < 0.05$) when compared to the FA control (–3675 ± 242 cm H_2O/s) (Figure 2B).

Compared to the FA group (13.2 ± 1.4 min), time to ischemic contracture was not affected in the O_3 group (14.0 ± 1.8 min), but was prolonged in the multi-pollutant-mixtures group during 20 min of ischemia (17.3 ± 0.5 min; $p < 0.05$). There was also an increase in post-ischemic recovery of LVDP at 40 min after reperfusion in the multi-pollutant-mixtures group compared to the FA control group (70.7 ± 23.7% for multi-pollutant-mixtures vs. 21.0 ± 7.1% for FA; $p = 0.05$) (Figure 3A). Infarct size was smaller in the multi-pollutant-mixtures group (39.6 ± 6.6%) compared to the FA control (57.3 ± 4.6% for FA; $p < 0.05$) (Figure 3B). By 40 min of reperfusion, HR, LVDP, contractility, and coronary flow rate were not different among the multi-pollutant-mixtures, O_3, and FA groups (Table 2).

Airway Inflammation

Compared to the FA controls, the BAL from mice exposed to the multi-pollutant-mixtures or O_3 showed an increase in the number of macrophages (Figure 4). However, there was no influx of neutrophils or other cell types measured after any exposure.

DISCUSSION

While our understanding of the health effects of single pollutants has advanced considerably over the last several decades, knowledge of the effects of multi-pollutant-mixtures is limited. In this study a photochemical reaction chamber was used to generate particle-free urban-like multi-pollutant-mixtures for the purpose of evaluating the pulmonary and cardiac responses in mice to inhalation of an atmosphere containing complex multi-pollutant-mixtures or O_3 at the same concentration as present in the multi-pollutant-mixtures. Thus, any difference in measured responses between these two exposures can be ascribed to effects of the multi-pollutant-mixtures. We show that short-term inhalation of photochemically altered particle-free multi-

pollutant-mixtures and O_3 alone depressed cardiac contractility (dP/dt) in isolated perfused heart. We also show that exposure to the particle-free multi-pollutant-mixtures delayed the onset of ischemic contracture and preserved contractile function during reperfusion in isolated mouse hearts. In addition, there is no significant difference of the mechanical responses between the multi-pollutant-mixtures and O_3 alone, but the magnitude of the effect of O_3 alone was always less than the effect of the multi-pollutant-mixtures. Thus, it appears that one or more of the multi-pollutant-mixture's component(s) contribute to an additional effect beyond that of O_3 alone.

Experimental studies implicate particulate mass and the physicochemical properties of air pollutants as determinants of the health effects of air pollution inhalation (Gordon 2007). In particular, organic components of air pollution are thought to play an important role in affecting biological responses (Castranova et al. 2001). In this study, mice were exposed to particle-free photochemically altered products of hydrocarbons and NO_x indicating that the cardiac effects measured following exposure resulted from gaseous components of multi-pollutant-mixtures. We have previously exposed cultured lung cells to similar multi-pollutant-mixtures in the photochemical reaction chamber and showed that gaseous products elicited biological and biochemical responses in the absence of particles (Sexton et al. 2004). Furthermore, animal studies have shown that spontaneously hypertensive and mildly cardiomyopathic rats exposed to filtered diesel exhaust exhibited either a similar degree or a greater magnitude of electrophysiological changes compared to whole diesel exhaust (Carll et al. 2012; Lamb et al. 2012), implying that gaseous components of air pollution might be driving the cardiovascular effects.

In this study, as the photochemical reaction progressed, levels of secondary chemical products such as O_3, formaldehyde, acetaldehyde and many volatile and semi-volatile organic hydrocarbons increase (Sexton et al. 2004). Therefore, animals were exposed to higher levels of secondary products compared to fresh urban mixtures, which may elicit biological effects on the cardiovascular system. It has been shown that secondary products formed during the photochemical reactions induced more robust inflammatory responses (Sexton et al. 2004) and greater genomic changes (Rager et al. 2011) in cultured lung cells. Those secondary products such as O_3 generated during the photochemical reactions could contribute to the biological effects, including the O_3-induced heart rate and baseline rate of left ventricular relaxation changes that we report in the present study. Epidemiological studies have linked short-term O_3 exposure with cardiovascular and respiratory mortality in 95 large US communities (Bell et al. 2004). In addition, O_3 exposure has also been associated with increased hospital admissions for heart failure (Yang 2008). Most recently, a controlled human exposure study has demonstrated that short-term O_3 exposure increased pulmonary and systemic inflammation, altered autonomic control of heart rhythm and induced changes in blood proteins involved in fibrinolysis (Devlin et al. 2012). In addition, an animal study has demonstrated that 5 days (8 hr/day) of 0.5 ppm O_3 exposure in mice altered heart rates and mean blood pressure, inhibited endothelial-dependent vasorelaxation, and induced mitochondrial damage and atherogenesis (Chuang et al. 2009).

The levels of secondary products formaldehyde and acetaldehyde were increased during the photochemical process in this study. Formaldehyde and acetaldehyde occur naturally in the environment and are produced in forest fires, automobile exhaust, and tobacco smoke. The ambient formaldehyde concentration was measured to be in the range of 0.4 to 7.5 ppb with a mean value of 2.2 ppb in New York City in the summer time in 2009 (Lin et al. 2012) and

ambient concentration of acetaldehyde averaged at 5 μg/m^3. In this study exposure to formaldehyde may have contributed to the observed cardio-depressive activity of the particle-free multi-pollutant-mixtures inhalation. Similarly, acetaldehyde, another component of the multi-pollutant-mixtures could affect the cardiac function as well. Acetaldehyde has been shown to disrupt Ca^{2+} handling in the myocardium and disturb cardiac excitation-contraction coupling (Oba et al. 2008) leading to reduced cardiac contractility. Therefore, the cardiac depressive effects of inhalation of the multi-pollutant-mixtures used in this study could have resulted from acetaldehyde induced myocardial toxicity. Above all, the respiratory responses of volatile and semi-volatile organic components of multi-pollutant-mixtures, such as O_3, have been investigated in the past; yet the cardiovascular effects of those components are not well known. More research is needed to evaluate the cardiovascular effects of exposure to these ubiquitous air contaminants.

The mechanistic basis for the cardiac mechanical effects of exposure to multi-pollutant-mixtures and O_3 is not explored in this study. However, there are several pathways by which the air pollution mixtures could affect the cardiovascular system (Brook et al. 2010). Oxidative stress from multi-pollutant-mixtures components such as O_3 could cause systemic oxidative stress, resulting in myocardial contractile dysfunction. In addition, activation of pulmonary receptors could initiate a neurocardiogenic effect producing an intracardiac response affecting cardiac cellular function. It is also possible that some components of multi-pollutant-mixtures might translocate into the circulation with attendant direct oxidative effects on the heart and vasculature. We only detected macrophage accumulation in BAL in this study, suggesting that airway inflammation may not mediate the cardiac effects of inhalation to multi-pollutant-mixtures or O_3.

The decreased LVDP and cardiac contractility, delayed ischemic contracture and preserved contractility during reperfusion consequent to the exposure to inhaled multi-pollutant-mixtures could indicate altered intracellular Ca^{2+} regulation in the myocardium. The pro-redox components of multi-pollutant-mixtures could modulate the cardiac myocytes Ca^{2+} handling by reducing the intracellular Ca^{2+}, or change in the sensitivity of the contractile proteins to Ca^{2+}, resulting in decreased cardiac contractility. On the other hand, the reduced intracellular Ca^{2+} overload during ischemia is associated with preservation of mitochondrial function and adenosine triphosphate stores (Kowalchuk and Nesto 1989), suggested by the multi-pollutant-mixtures induced delay in ischemic contracture and better recovery of post-ischemic LVDP and smaller infarct size during reperfusion. Future studies are needed to better understand the role of intracellular Ca^{2+} regulation in mediating cardiovascular physiological effects from air pollution exposure.

In this study we used a perfused isolated heart model to evaluate the ventricular function. However, isolated heart models lack innervation and blood supply and therefore lack influence by autonomic and hormonal control, which could alter findings in intact hearts.

CONCLUSION

We show that inhalation of particle-free photochemically altered multi-pollutant-mixtures affects cardiac function in isolated mouse hearts. Specifically, inhalation of multi-pollutant-mixtures depressed cardiac contractility in isolated non-ischemic hearts, delayed ischemic contracture and preserved cardiac contractility in reperfused hearts, while eliciting mild pulmonary inflammation evidenced only by macrophage accumulation. Future studies are needed to identify the active

components of photochemical reaction products responsible for the effects on myocardial mechanical performance, and the mechanisms that underlie the cardiac effects.

REFERENCES

Bell ML, McDermott A, Zeger SL, Samet JM, Dominici F. 2004. Ozone and short-term mortality in 95 US urban communities, 1987-2000. JAMA. 292(19):2372-2378.

Brook RD, Rajagopalan S, Pope CA, 3rd, Brook JR, Bhatnagar A, Diez-Roux AV, et al. 2010. Particulate matter air pollution and cardiovascular disease: An update to the scientific statement from the American Heart Association. Circulation. 121(21):2331-2378.

Carll AP, Hazari MS, Perez CM, Krantz QT, King CJ, Winsett DW, et al. 2012. Whole and particle-free diesel exhausts differentially affect cardiac electrophysiology, blood pressure, and autonomic balance in heart failure-prone rats. Toxicol Sci. 128(2):490-499.

Castranova V, Ma JY, Yang HM, Antonini JM, Butterworth L, Barger MW, et al. 2001. Effect of exposure to diesel exhaust particles on the susceptibility of the lung to infection. Environ Health Perspect. 109 Suppl 4:609-612.

Cho SH, Tong H, McGee JK, Baldauf RW, Krantz QT, Gilmour MI. 2009. Comparative toxicity of size-fractionated airborne particulate matter collected at different distances from an urban highway. Environ Health Perspect. 117(11):1682-1689.

Chuang GC, Yang Z, Westbrook DG, Pompilius M, Ballinger CA, White CR, et al. 2009. Pulmonary ozone exposure induces vascular dysfunction, mitochondrial damage, and atherogenesis. Am J Physiol Lung Cell Mol Physiol. 297(2):L209-216.

Cozzi E, Hazarika S, Stallings HW, 3rd, Cascio WE, Devlin RB, Lust RM, et al. 2006. Ultrafine particulate matter exposure augments ischemia-reperfusion injury in mice. Am J Physiol Heart Circ Physiol. 291(2):H894-903.

Devlin RB, Duncan KE, Jardim M, Schmitt MT, Rappold AG, Diaz-Sanchez D. 2012. Controlled exposure of healthy young volunteers to ozone causes cardiovascular effects. Circulation. 126(1):104-111.

Dominici F, Peng RD, Barr CD, Bell ML. 2010. Protecting human health from air pollution: shifting from a single-pollutant to a multipollutant approach. Epidemiology. 21(2):187-194.

Gordon T. 2007. Linking health effects to PM components, size, and sources. Inhal Toxicol. 19 Suppl 1:3-6.

Jeffries H. 1995. Photochemical air pollution. In Composition, chemistry, and climate of the atmosphere, ed., H.B. Singh, pp.308-348. New York: Van Nostrand-Reinhold.

Kanakidou M, J. H. Seinfeld, S. N. Pandis, I. Barnes, F. J. Dentener, M. C. Facchini, et al. 2005. Organic aerosol and global climate modelling: a review. Atmos Chem Phys 5:1053–1123.

Kowalchuk GJ, Nesto RW. 1989. Calcium antagonists and myocardial protection. Am J Cardiol. 64(11):10F-17F.

Lamb CM, Hazari MS, Haykal-Coates N, Carll AP, Krantz QT, King C, et al. 2012. Divergent electrocardiographic responses to whole and particle-free diesel exhaust inhalation in spontaneously hypertensive rats. Toxicol Sci. 125(2):558-568.

Lin Y, Schwab JJ, Demerjian KL, Bae MS, Chen WN, Sun Y, et al. 2012. Summertime formaldehyde observations in New York City: Ambient levels, sources and its contribution to HOx radicals. J Geophys Res. 117:D08305.

Oba T, Maeno Y, Nagao M, Sakuma N, Murayama T. 2008. Cellular redox state protects acetaldehyde-induced alteration in cardiomyocyte function by modifying Ca2+ release from sarcoplasmic reticulum. Am J Physiol Heart Circ Physiol. 294(1):H121-133.

Rager JE, Lichtveld K, Ebersviller S, Smeester L, Jaspers I, Sexton KG, et al. 2011. A toxicogenomic comparison of primary and photochemically altered air pollutant mixtures. Environ Health Perspect. 119(11):1583-1589.

Sexton KG, Jeffries HE, Jang M, Kamens RM, Doyle M, Voicu I, et al. 2004. Photochemical products in urban mixtures enhance inflammatory responses in lung cells. Inhal Toxicol. 16 Suppl 1:107-114.

Tong H, Cheng WY, Samet JM, Gilmour MI, Devlin RB. 2010. Differential cardiopulmonary effects of size-fractionated ambient particulate matter in mice. Cardiovasc Toxicol. 10(4):259-267.

Tong H, McGee JK, Saxena RK, Kodavanti UP, Devlin RB, Gilmour MI. 2009. Influence of acid functionalization on the cardiopulmonary toxicity of carbon nanotubes and carbon black particles in mice. Toxicol Appl Pharmacol. 239(3):224-232.

Yang CY. 2008. Air pollution and hospital admissions for congestive heart failure in a subtropical city: Taipei, Taiwan. J Toxicol Environ Health A. 71(16):1085-1090

Table 1. Average concentrations (ppb) of compounds measured in the photoreaction chamber.

Chemicals	Pollutant Exposure		
	Multi-pollutant-mixtures	O_3	Filtered Air
O_3	243	245	10
NO	0	0	1
NO_2	18	4	7
CO	500	300	300
Peroxyacetyl nitrate	1.3	< 0.2	< 0.2
Formaldehyde	64.2	12.2	12.2
Acetaldehyde	22.1	0	0
Acetone	11.1	0	0
2-Hexanone	17.1	0	0
Glyoxal	11.7	0.6	0.6
Methylglyoxal	15.7	0.8	0.8
Unknown cluster	74.9	9.5	9.5
Estimated total carbonyls	286.0	23.1	23.1
Total organic precursors	2000	< 20	< 20
Alkanes, 23 total	1133		
Isopentane	173		
n-Butane	147		
Propane	92		
Ethane	77		
Alkenes, 16 total	262		
Ethene	53		
2,3,3-Trimethyl-1-butene	32		
c-2-Pentene	27		
t-2-Butene	23		
Aromatics, 13 total	605		
Toluene	138		
1,2,4-Trimethylbenezene	112		
m-Xylene	74		
Benzene	44		

O_3, NO, NO_2, and CO was measured every min during the exposure period and averaged for reporting. The other compounds were measured every 15 min during the exposure period. All samples were measured by gas chromatography and mass spectrometry, using a direct cryogenically trapped sample taken from the chamber. Since the exposures were conducted in the dark, concentrations were stable and homogeneous.

Table 2. Hemodynamic measures of isolated mouse hearts.

Experimental Groups	Filtered Air (*n* = 8)	O_3 (*n* = 6)	Mixtures (*n* = 7)
Baseline condition			
HR (bpm)	331 ± 10	280 ± 10*	286 ± 30
LVDP (cmH_2O)	146.4 ± 14.8	134.2 ± 25.4	69.2 ± 16.0*
dP/dt_{max} (cmH_2O/sec)	5405 ± 400	4070 ± 704	2764 ± 558*
dP/dt_{min} (cmH_2O/sec)	–3675 ± 242	–2477 ± 407*	–1822 ± 335**
Coronary flow rate (mL/min)	2.0 ± 0.2	1.6 ± 0.3	1.8 ± 0.2
At 40 min of reperfusion			
HR (bpm)	290 ± 21	278 ± 28	280 ± 23
LVDP (cmH_2O)	33.5 ± 11.4	86.5 ± 34.9	38.0 ± 8.8
dP/dt_{max} (cmH_2O/sec)	1418 ± 467	2963 ± 1212	1612 ± 352
dP/dt_{min} (cmH_2O/sec)	–801 ± 257	–1751 ± 674	–872 ± 150
Coronary flow rate (mL/min)	1.6 ± 0.1	1.2 ± 0.2	1.5 ± 0.2

Values are means ± SE. HR = heart rate, LVDP = left ventricular developed pressure; dP/dt_{max} = maximum 1st derivative of the change in left ventricular pressure/time; dP/dt_{min} = minimum 1st derivative of the change in left ventricular pressure/time. Kruskal-Wallis U-test followed by Dunn's multiple comparison was used to compare differences among the multi-pollutant-mixtures, O_3, and FA control group. *$p < 0.05$ compared with FA control group; **$p < 0.01$ compared with FA control group.

FIGURE LEGENDS

Figure 1. Heart rate and cardiac function in isolated perfused murine hearts before ischemia. Heart rate (**A**) and left ventricular developed pressure (LVDP) (**B**) at baseline prior to ischemia in murine hearts isolated 8 to 11 hr after inhalation exposure to filtered air (FA), multi-pollutant mixtures (mixtures), or O_3 for 4 hr as described in METHODS. n = 8 in the FA group, n=6 in the O_3 group, and n=7 in the mixtures group. Kruskal-Wallis U-test followed by Dunn's multiple comparison was used to compare differences among the mixtures, O_3, and FA control group. *p < 0.05; compared with FA control group.

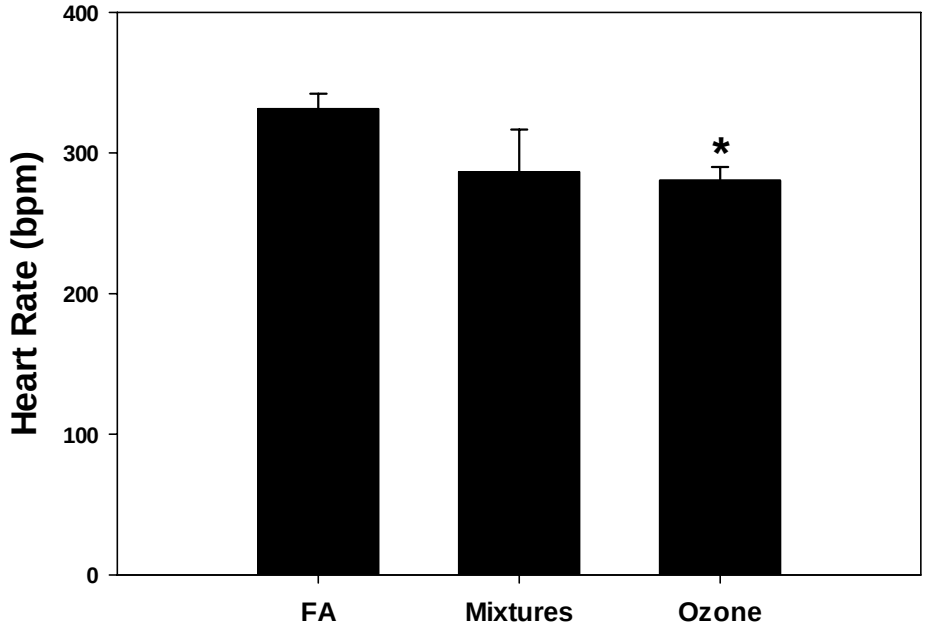
Figure 2. Multi-pollutant-mixtures and O_3 inhalation reduced cardiac contractility in isolated, perfused murine hearts. The cardiac contractility assessed by maximum (**A**) and minimum (**B**) dP/dt at baseline prior to ischemia in murine hearts isolated 8 hr after inhalation exposure to filtered air (FA), multi-pollutant mixtures (mixtures), or O_3 for 4 hr as described in METHODS. n = 8 in the FA group, n=6 in the O_3 group, and n=7 in the mixtures group. Kruskal-Wallis U-test followed by Dunn's multiple comparison was used to compare differences among the mixtures, O_3, and FA control group. *p < 0.05 and **p<0.01 compared with FA control group.

Figure 3. Multi-pollutant-mixtures exposure improved recovery of post-ischemic cardiac function and reduced infarct size in murine hearts. **A**. Recovery of left ventricular developed pressure (LVDP), expressed as a percentage of the initial baseline pre-ischemic LVDP was measured after 20 min of ischemia and 40 min of reperfusion in mouse hearts isolated 8 hr after inhalation exposure to filtered air (FA), multi-pollutant mixtures (mixtures), or O_3 for 4 hr as described in METHODS. **B**. Infarct size, expressed as a percentage of the total ventricular

section was measured after 20 min of ischemia and 2 hr of reperfusion as described in METHODS. n = 8 in the FA group, n=6 in the O_3 group, and n=7 in the mixtures group. Kruskal-Wallis U-test followed by Dunn's multiple comparison was used to compare differences among the mixtures, O_3, and FA control group. *$p < 0.05$; compared with FA control group.

Figure 4. Multi-pollutant-mixtures and O_3 exposure increased macrophages number in the bronchoalveolar lavage (BAL) fluid. Macrophages number in the bronchoalveolar lavage fluid (BAL) 12 hr after exposure to filtered air (FA), multi-pollutant mixtures (mixtures), or O_3 for 4 hr as described in METHODS. n = 8 in each group. Kruskal-Wallis U-test followed by Dunn's multiple comparison was used to compare differences among the mixtures, O_3, and FA control group. *$p < 0.05$; compared with FA control group.

A


400
300
200
100
0
Heart Rate (bpm)
*
FA
Mixtures
Ozone

B

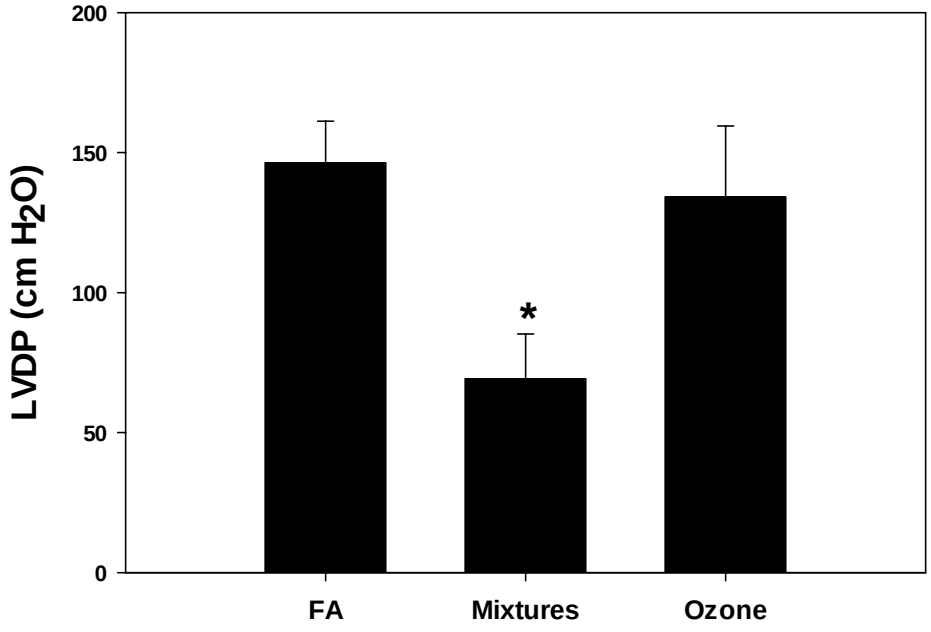
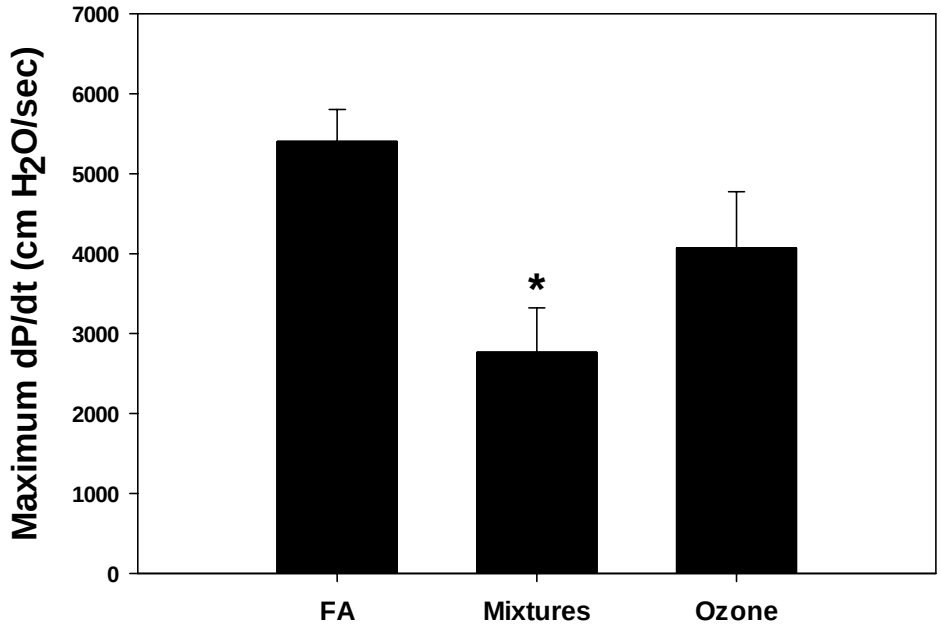

200
150
100
50
0
LVDP (cm H2O)
*
FA
Mixtures
Ozone

Figure 1

A


Maximum dP/dt (cm H2O/sec)
7000
6000
5000
4000
3000
2000
1000
0
*
FA
Mixtures
Ozone

B

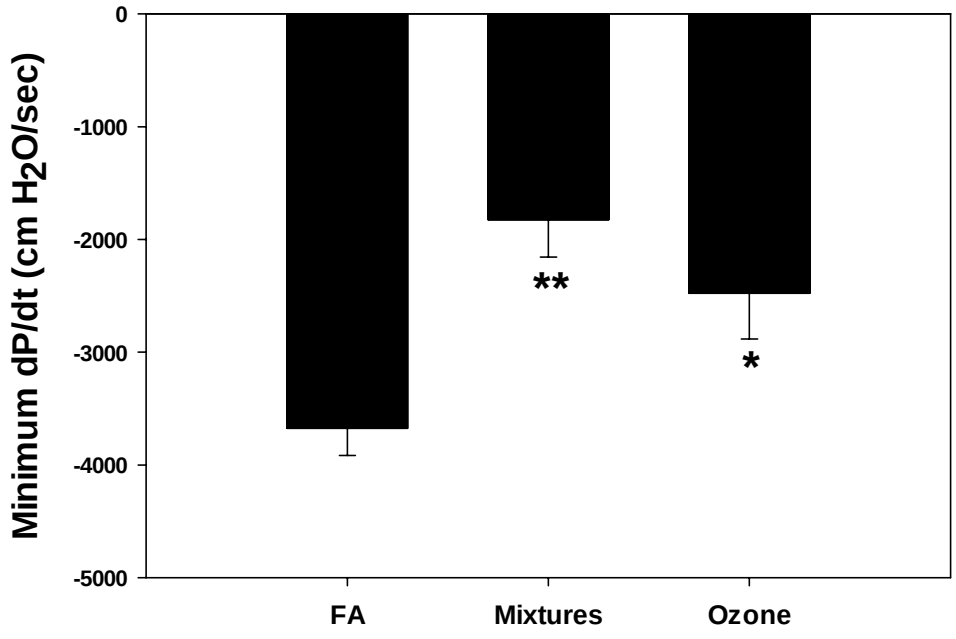
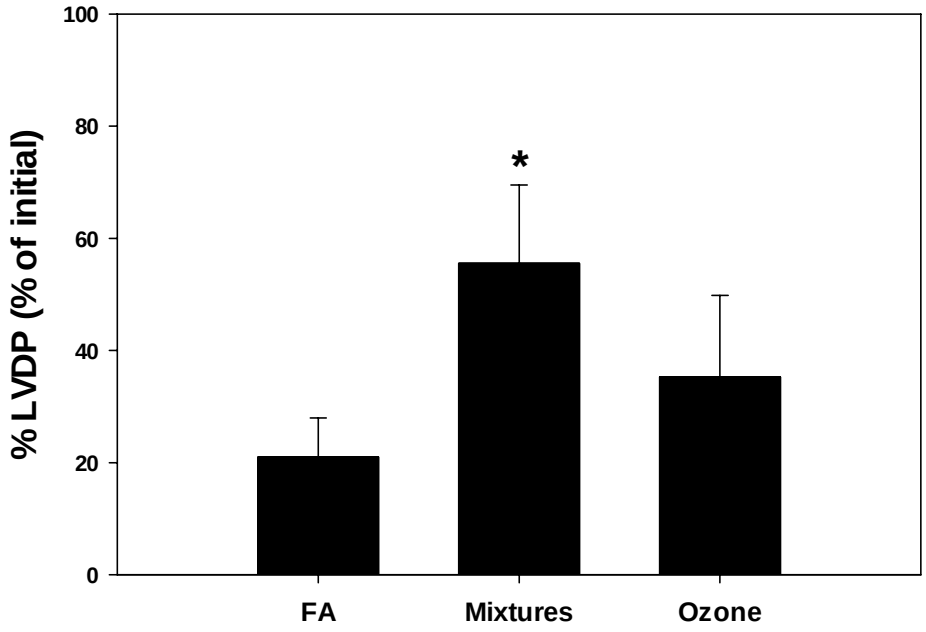

Minimum dP/dt (cm H2O/sec)
0
-1000
-2000
-3000
-4000
-5000
**
*
FA
Mixtures
Ozone

Figure 2

A


100
80
60
40
20
0
% LVDP (% of initial)
*
FA
Mixtures
Ozone

B

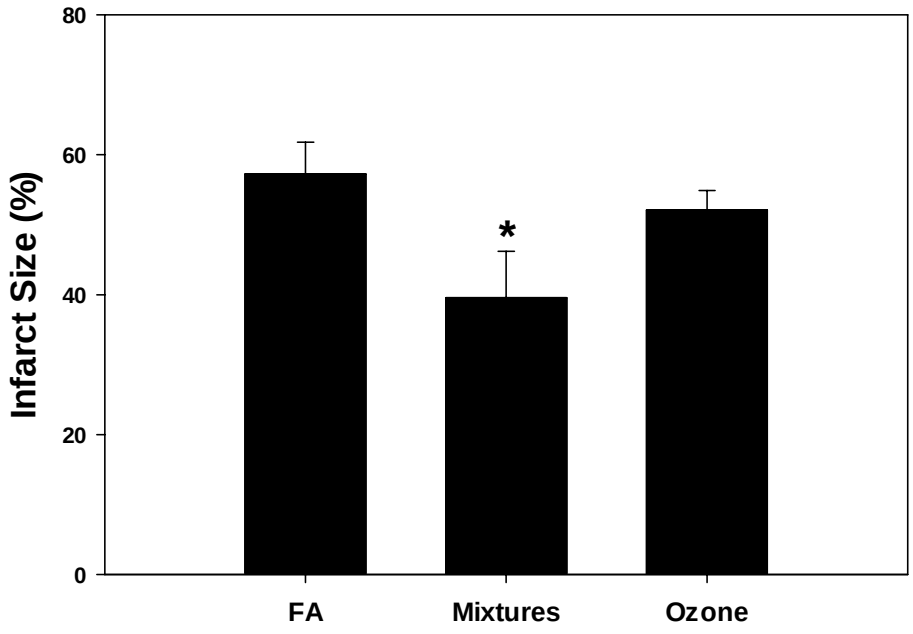

80
60
40
20
0
Infarct Size (%)
*
FA
Mixtures
Ozone

Figure 3

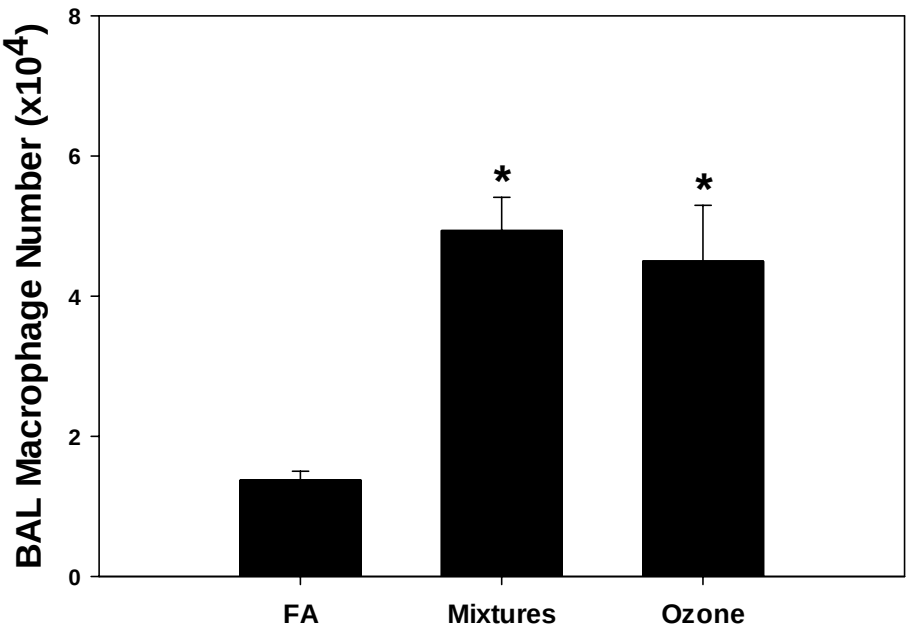


Figure 4